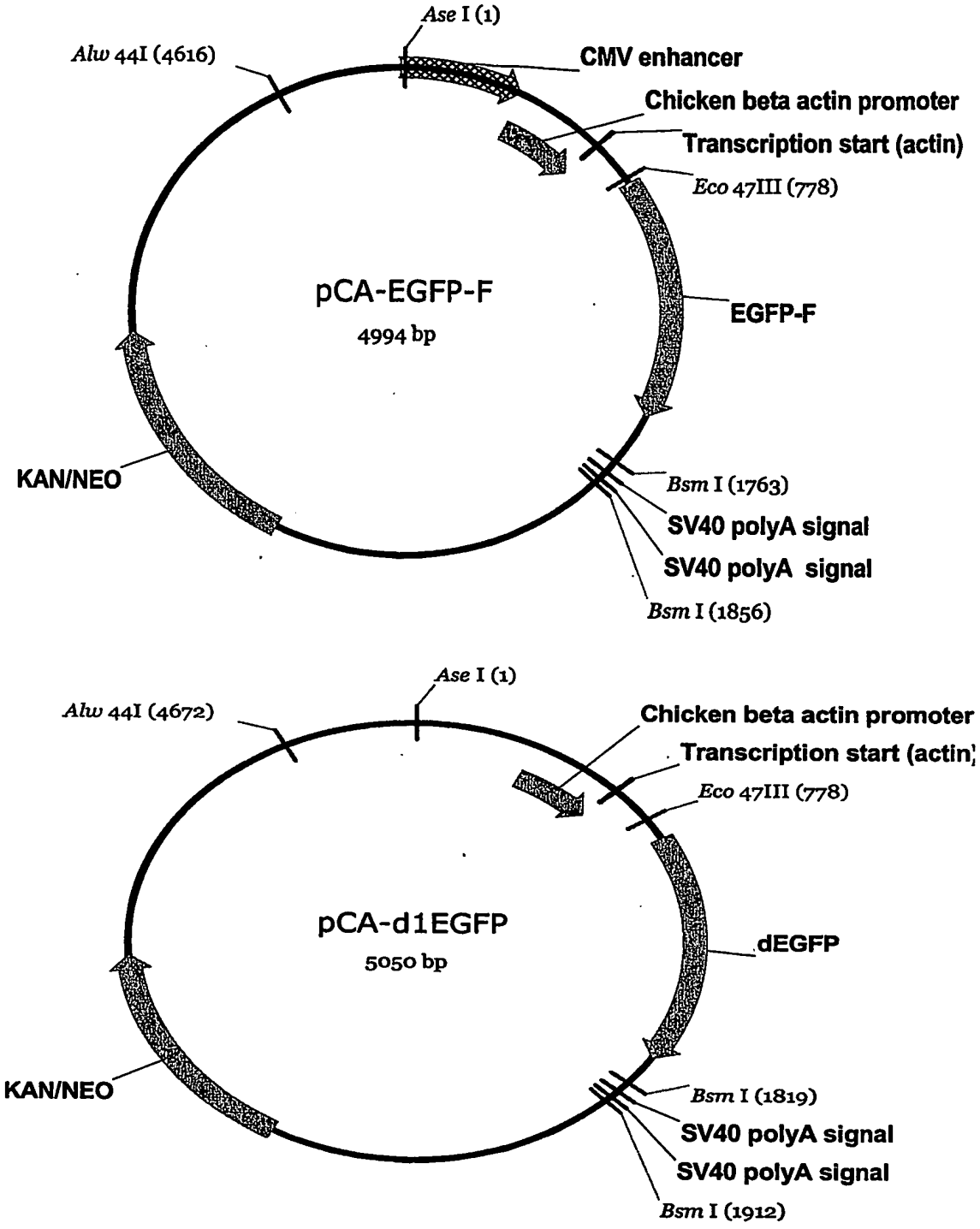

Ase I (1)
Alw 44I (4616)
CMV enhancer
Chicken beta actin promoter
Transcription start (actin)
Eco 47III (778)
pCA-EGFP-F
4994 bp
EGFP-F
KAN/NEO
Bsm I (1763)
SV40 polyA signal
SV40 polyA signal
Bsm I (1856)
Ase I (1)
Alw 44I (4672)
Chicken beta actin promoter
Transcription start (actin)
Eco 47III (778)
pCA-d1EGFP
5050 bp
dEGFP
KAN/NEO
Bsm I (1819)
SV40 polyA signal
SV40 polyA signal
Bsm I (1912)

Fig. 1

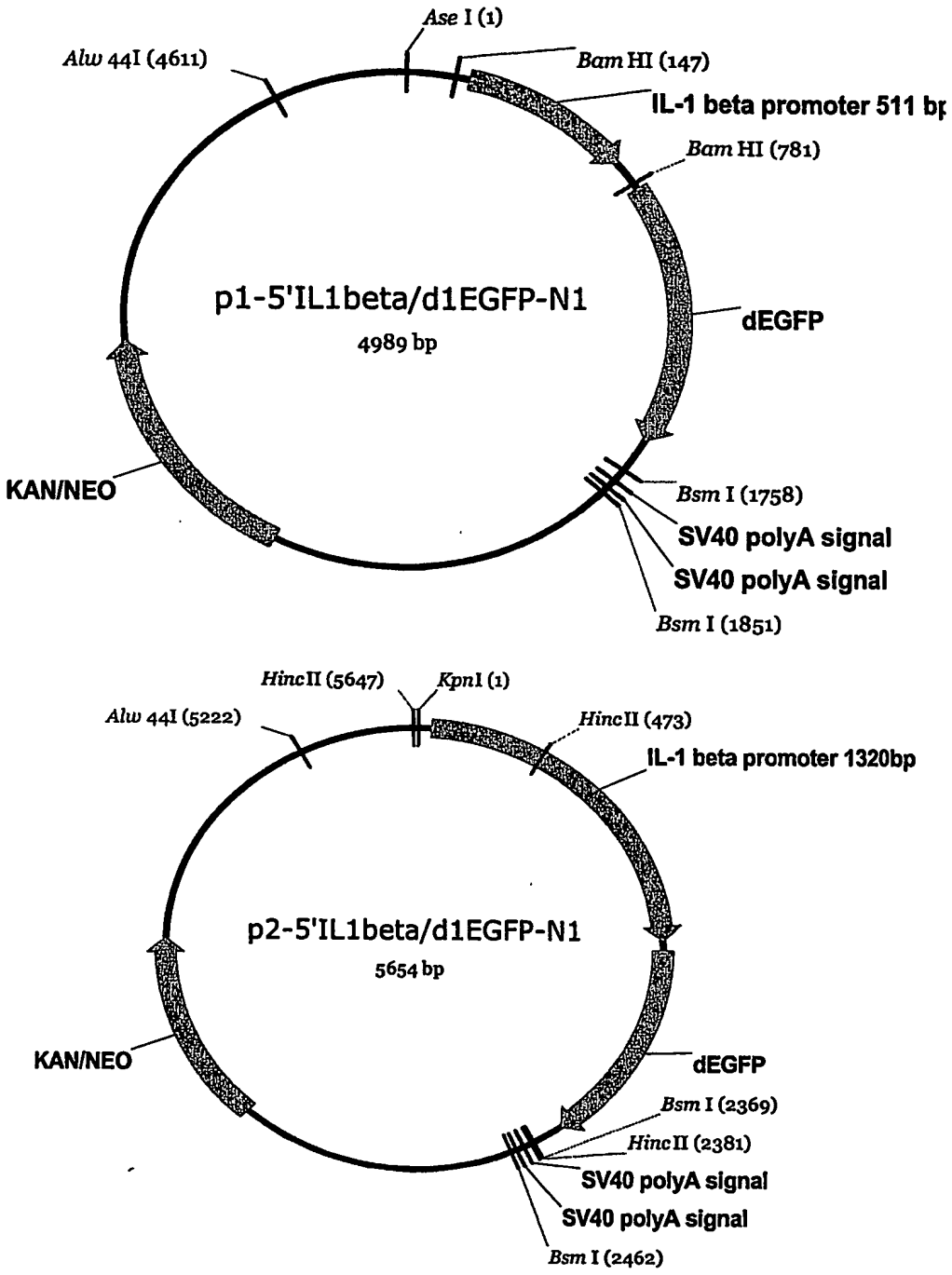

Ase I (1)
Alw 44I (4611)
Bam HI (147)
IL-1 beta promoter 511 bp
Bam HI (781)
p1-5'IL1beta/d1EGFP-N1
4989 bp
dEGFP
KAN/NEO
Bsm I (1758)
SV40 polyA signal
SV40 polyA signal
Bsm I (1851)
HincII (5647)
KpnI (1)
Alw 44I (5222)
HincII (473)
IL-1 beta promoter 1320bp
p2-5'IL1beta/d1EGFP-N1
5654 bp
KAN/NEO
dEGFP
Bsm I (2369)
HincII (2381)
SV40 polyA signal
SV40 polyA signal
Bsm I (2462)

Fig. 2

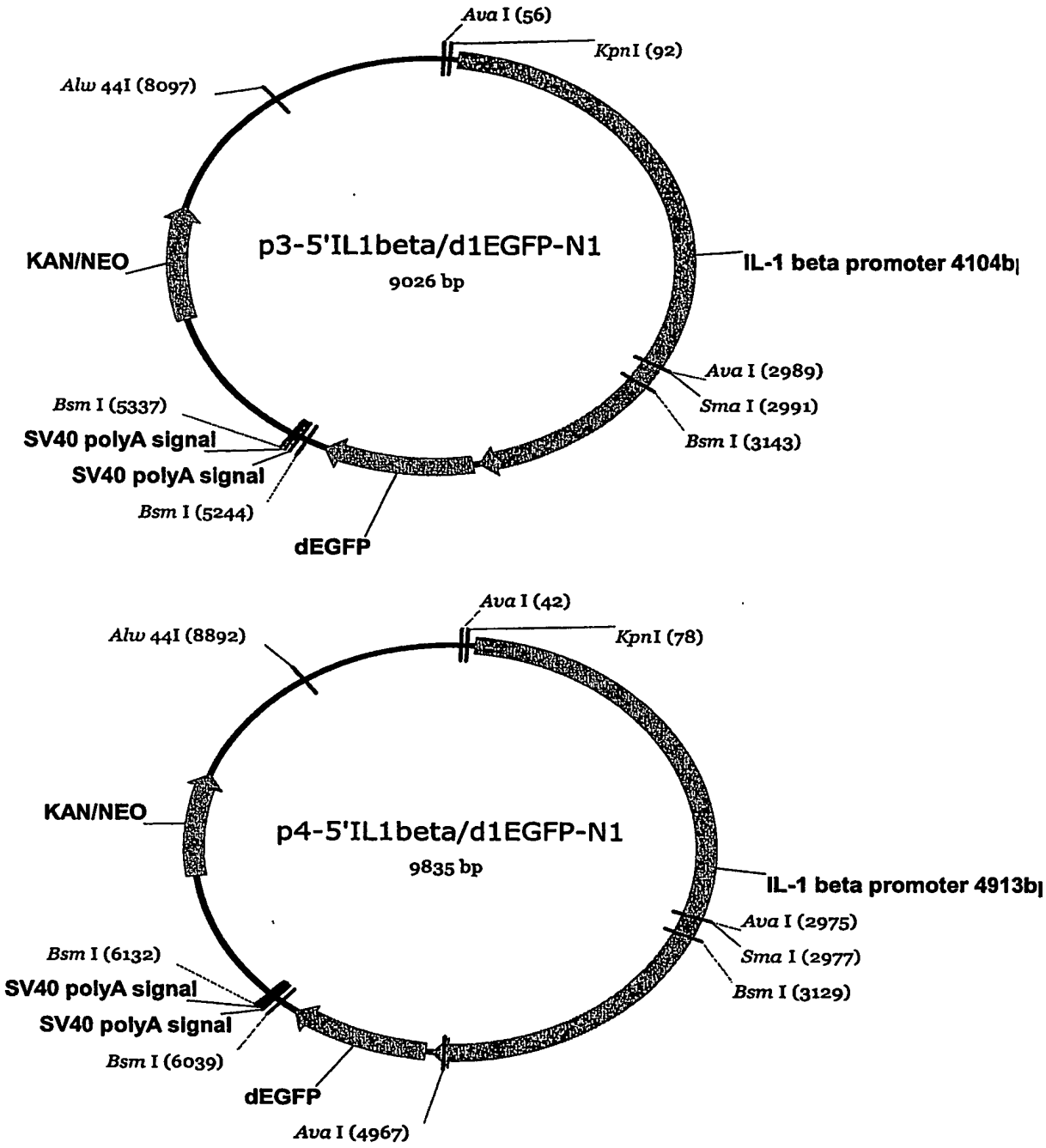

Ava I (56)
KpnI (92)
Alw 44I (8097)
p3-5'IL1beta/d1EGFP-N1
9026 bp
KAN/NEO
IL-1 beta promoter 4104b
Ava I (2989)
Sma I (2991)
Bsm I (3143)
Bsm I (5337)
SV40 polyA signal
SV40 polyA signal
Bsm I (5244)
dEGFP
Ava I (42)
KpnI (78)
Alw 44I (8892)
p4-5'IL1beta/d1EGFP-N1
9835 bp
KAN/NEO
IL-1 beta promoter 4913b
Ava I (2975)
Sma I (2977)
Bsm I (3129)
Bsm I (6132)
SV40 polyA signal
SV40 polyA signal
Bsm I (6039)
dEGFP
Ava I (4967)

Fig. 3

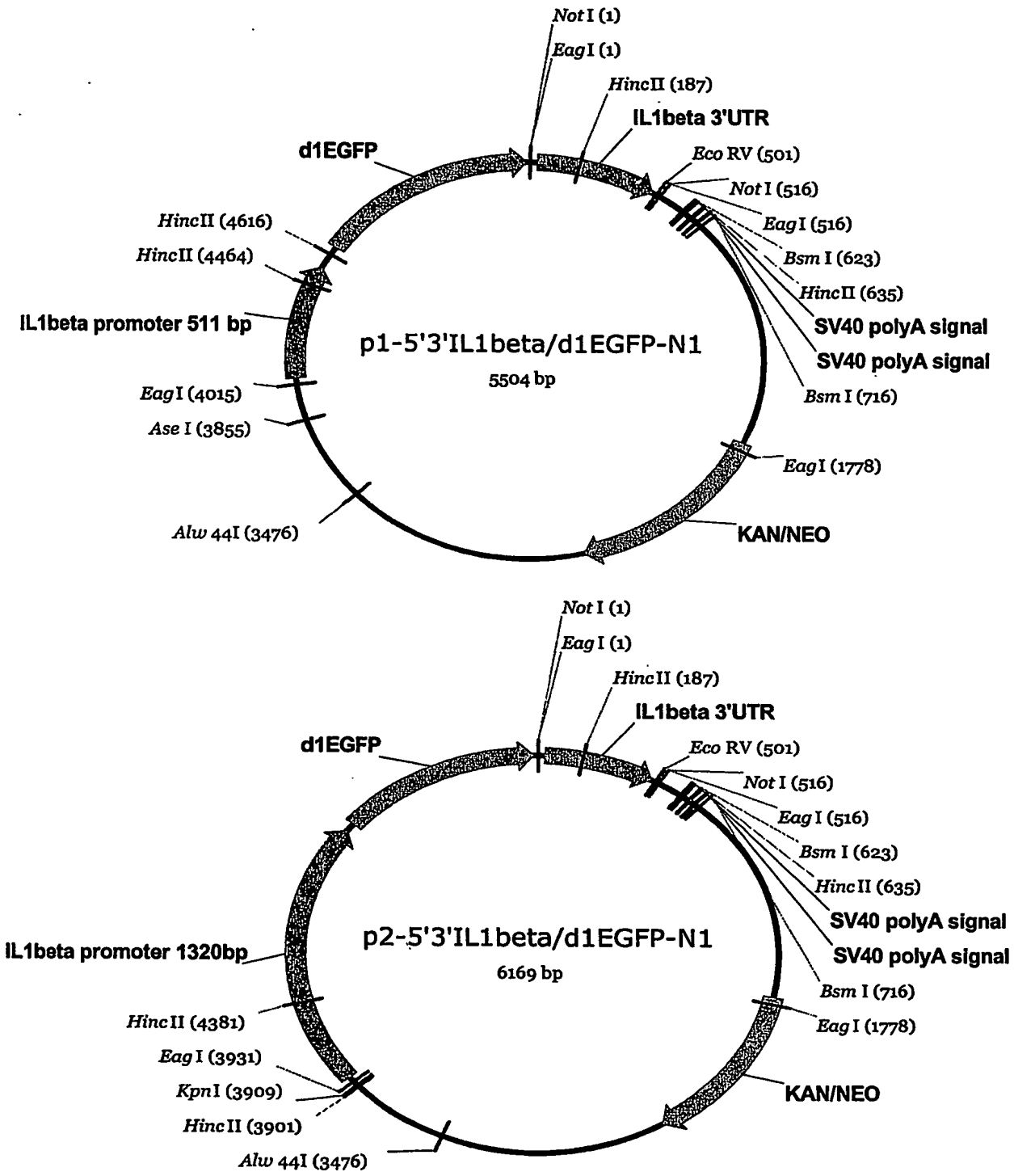

Not I (1)
Eag I (1)
HincII (187)
IL1beta 3'UTR
d1EGFP
Eco RV (501)
Not I (516)
Eag I (516)
Bsm I (623)
HincII (635)
SV40 polyA signal
SV40 polyA signal
HincII (4616)
HincII (4464)
IL1beta promoter 511 bp
p1-5'3'IL1beta/d1EGFP-N1
5504 bp
Eag I (4015)
Ase I (3855)
Bsm I (716)
Eag I (1778)
Alw 44I (3476)
KAN/NEO
Not I (1)
Eag I (1)
HincII (187)
IL1beta 3'UTR
d1EGFP
Eco RV (501)
Not I (516)
Eag I (516)
Bsm I (623)
HincII (635)
SV40 polyA signal
SV40 polyA signal
IL1beta promoter 1320bp
p2-5'3'IL1beta/d1EGFP-N1
6169 bp
Bsm I (716)
HincII (4381)
Eag I (1778)
Eag I (3931)
Kpn I (3909)
KAN/NEO
HincII (3901)
Alw 44I (3476)

Fig. 4

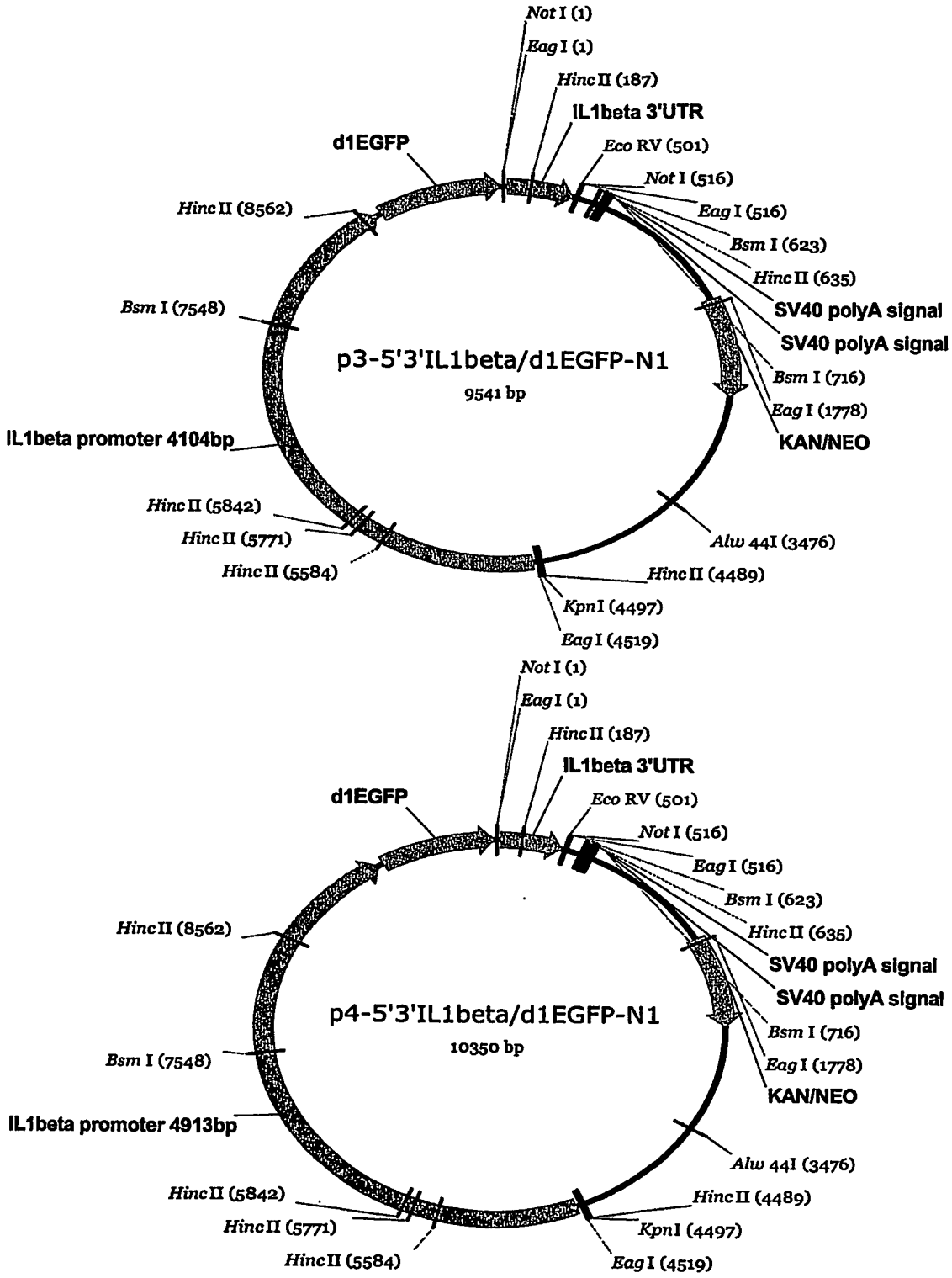

Not I (1)
Eag I (1)
Hinc II (187)
IL1beta 3'UTR
d1EGFP
Eco RV (501)
Not I (516)
Hinc II (8562)
Eag I (516)
Bsm I (623)
Hinc II (635)
Bsm I (7548)
SV40 polyA signal
SV40 polyA signal
p3-5'3'IL1beta/d1EGFP-N1
9541 bp
Bsm I (716)
Eag I (1778)
IL1beta promoter 4104bp
KAN/NEO
Hinc II (5842)
Hinc II (5771)
Alw 44I (3476)
Hinc II (5584)
Hinc II (4489)
Kpn I (4497)
Eag I (4519)
Not I (1)
Eag I (1)
Hinc II (187)
IL1beta 3'UTR
d1EGFP
Eco RV (501)
Not I (516)
Eag I (516)
Bsm I (623)
Hinc II (8562)
Hinc II (635)
SV40 polyA signal
SV40 polyA signal
p4-5'3'IL1beta/d1EGFP-N1
10350 bp
Bsm I (716)
Bsm I (7548)
Eag I (1778)
KAN/NEO
IL1beta promoter 4913bp
Alw 44I (3476)
Hinc II (5842)
Hinc II (4489)
Hinc II (5771)
Kpn I (4497)
Hinc II (5584)
Eag I (4519)

Fig. 5

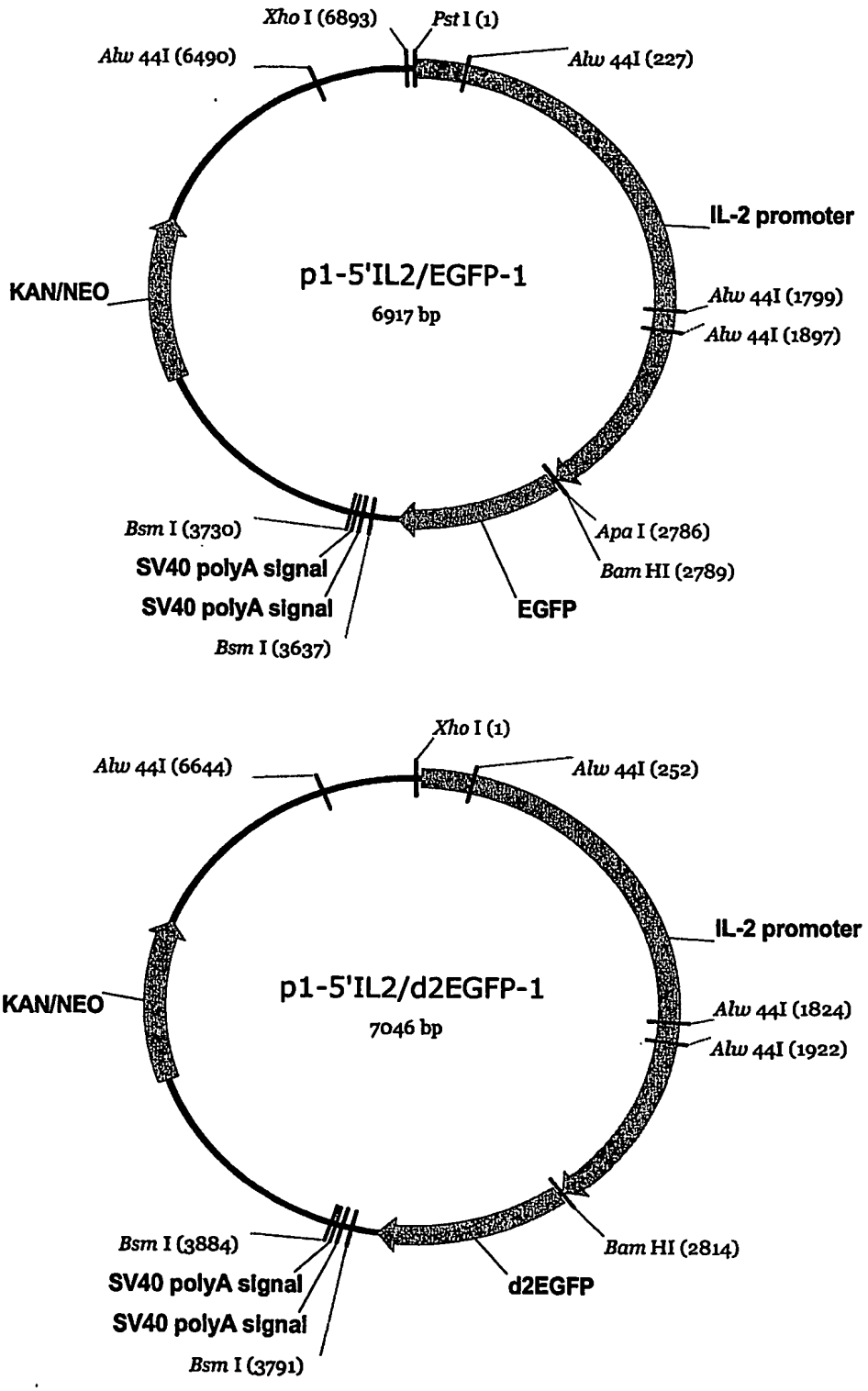

Xho I (6893)
Pst I (1)
Alw 44I (6490)
Alw 44I (227)
IL-2 promoter
p1-5'IL2/EGFP-1
6917 bp
KAN/NEO
Alw 44I (1799)
Alw 44I (1897)
Bsm I (3730)
Apa I (2786)
SV40 polyA signal
Bam HI (2789)
SV40 polyA signal
EGFP
Bsm I (3637)
Xho I (1)
Alw 44I (6644)
Alw 44I (252)
IL-2 promoter
p1-5'IL2/d2EGFP-1
7046 bp
KAN/NEO
Alw 44I (1824)
Alw 44I (1922)
Bsm I (3884)
Bam HI (2814)
SV40 polyA signal
d2EGFP
SV40 polyA signal
Bsm I (3791)

Fig. 6

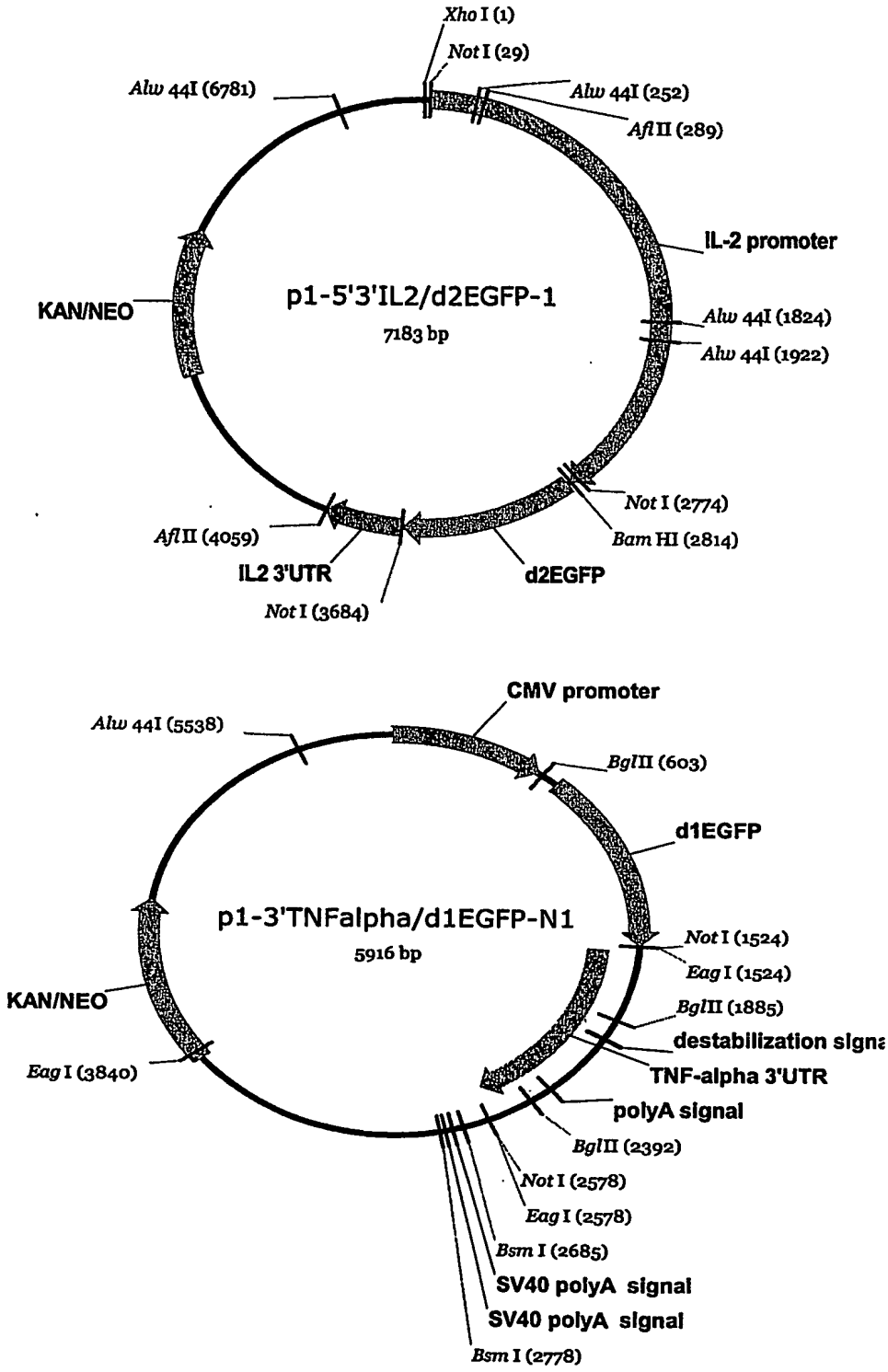

Xho I (1)
Not I (29)
Alw 44I (252)
Afl II (289)
IL-2 promoter
Alw 44I (1824)
Alw 44I (1922)
Not I (2774)
Bam HI (2814)
d2EGFP
Not I (3684)
Afl II (4059)
IL2 3'UTR
KAN/NEO
Alw 44I (6781)
p1-5'3'IL2/d2EGFP-1
7183 bp
CMV promoter
Bgl II (603)
d1EGFP
Not I (1524)
Eag I (1524)
Bgl II (1885)
destabilization signa
TNF-alpha 3'UTR
polyA signal
Bgl II (2392)
Not I (2578)
Eag I (2578)
Bsm I (2685)
SV40 polyA signal
SV40 polyA signal
Bsm I (2778)
Eag I (3840)
KAN/NEO
Alw 44I (5538)
p1-3'TNFalpha/d1EGFP-N1
5916 bp

Fig. 7

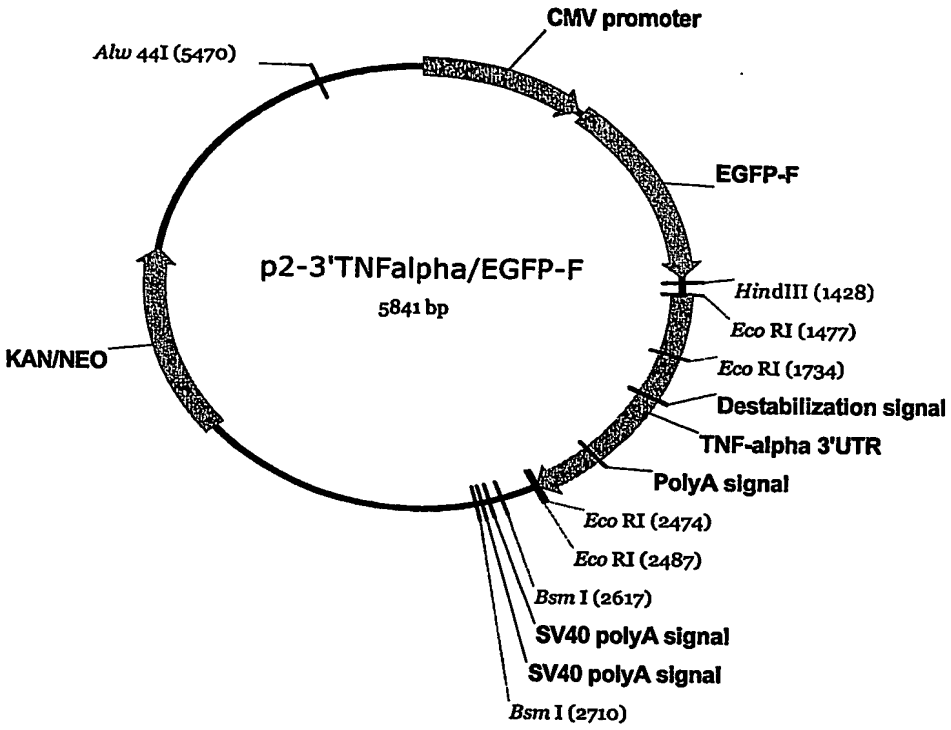

CMV promoter
Alw 44I (5470)
EGFP-F
p2-3'TNFalpha/EGFP-F
5841 bp
HindIII (1428)
Eco RI (1477)
Eco RI (1734)
KAN/NEO
Destabilization signal
TNF-alpha 3'UTR
PolyA signal
Eco RI (2474)
Eco RI (2487)
Bsm I (2617)
SV40 polyA signal
SV40 polyA signal
Bsm I (2710)

Fig. 8

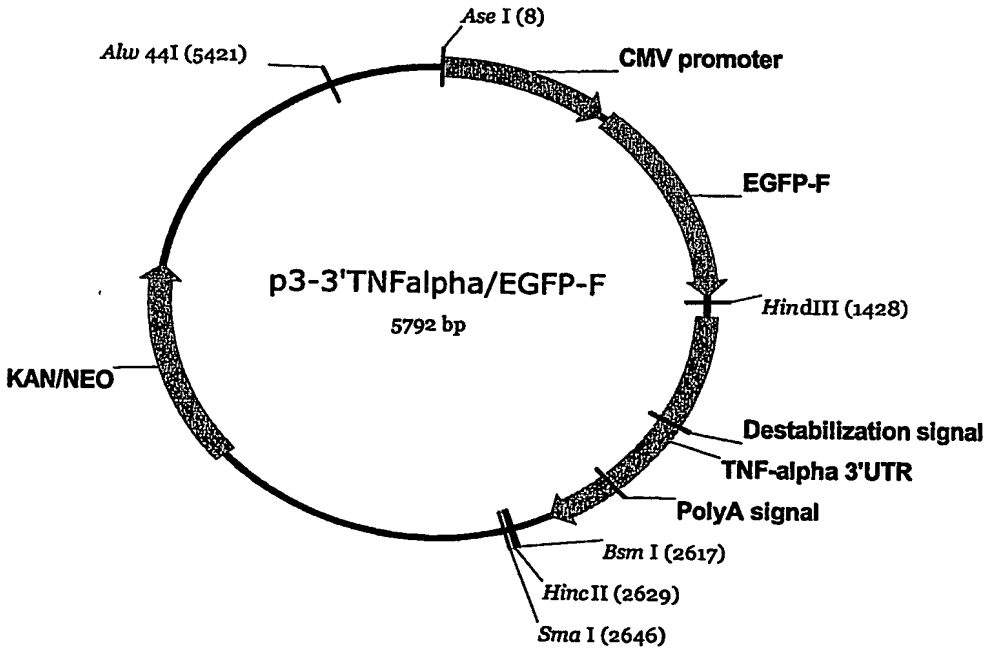

Ase I (8)
Alw 44I (5421)
CMV promoter
EGFP-F
p3-3'TNFalpha/EGFP-F
5792 bp
HindIII (1428)
KAN/NEO
Destabilization signal
TNF-alpha 3'UTR
PolyA signal
Bsm I (2617)
HincII (2629)
Sma I (2646)

Fig. 9

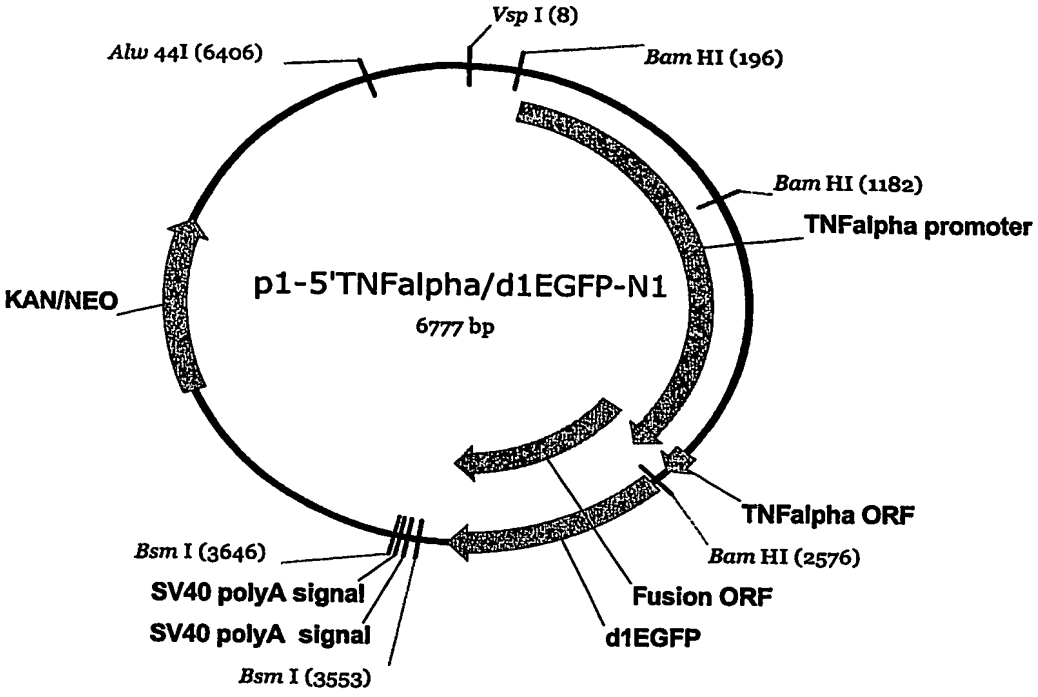

Vsp I (8)
Alw 44I (6406)
Bam HI (196)
Bam HI (1182)
TNFalpha promoter
KAN/NEO
p1-5'TNFalpha/d1EGFP-N1
6777 bp
TNFalpha ORF
Bam HI (2576)
Bsm I (3646)
SV40 polyA signal
SV40 polyA signal
Fusion ORF
d1EGFP
Bsm I (3553)

Fig. 10

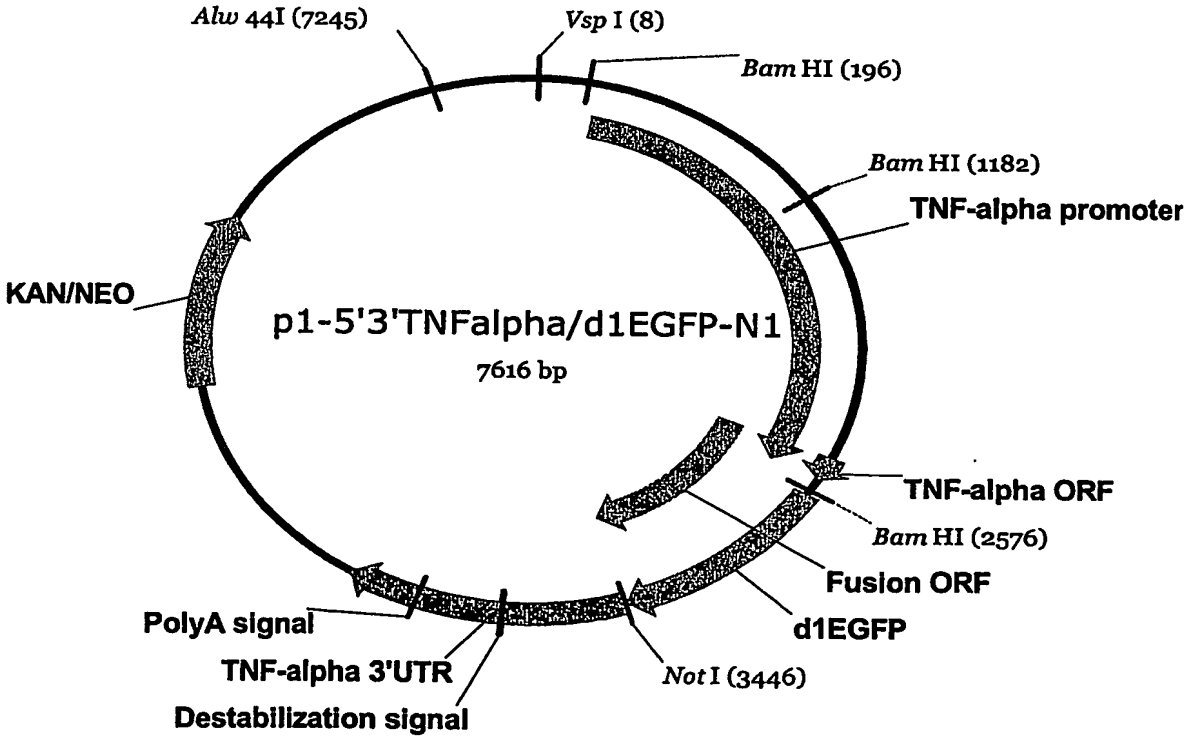

Alw 44I (7245)
Vsp I (8)
Bam HI (196)
Bam HI (1182)
TNF-alpha promoter
KAN/NEO
p1-5'3'TNFalpha/d1EGFP-N1
7616 bp
TNF-alpha ORF
Bam HI (2576)
Fusion ORF
d1EGFP
PolyA signal
TNF-alpha 3'UTR
Destabilization signal
Not I (3446)

Fig. 11

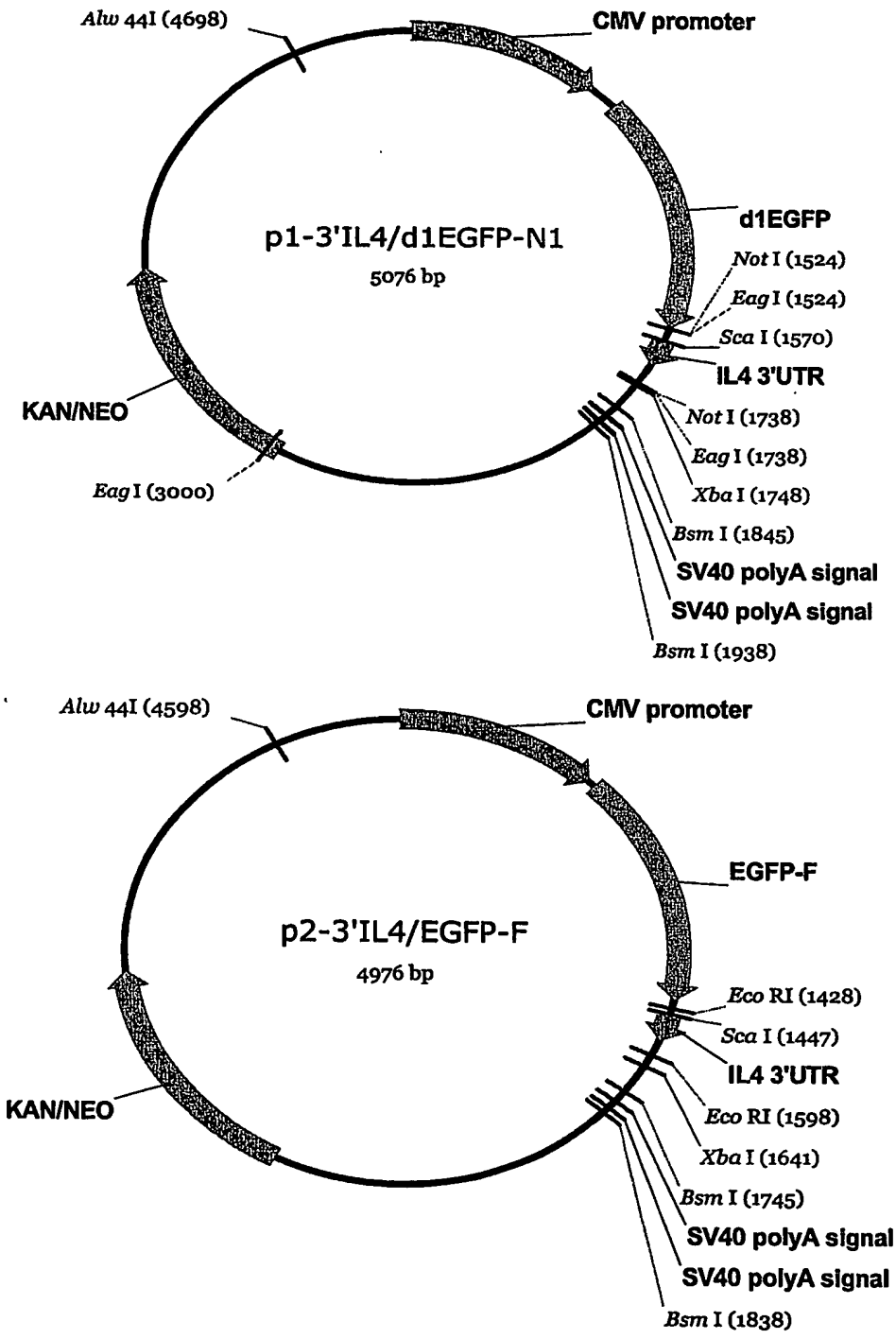

Alw 44I (4698)
CMV promoter
p1-3'IL4/d1EGFP-N1
5076 bp
d1EGFP
Not I (1524)
Eag I (1524)
Sca I (1570)
IL4 3'UTR
KAN/NEO
Not I (1738)
Eag I (1738)
Xba I (1748)
Eag I (3000)
Bsm I (1845)
SV40 polyA signal
SV40 polyA signal
Bsm I (1938)
Alw 44I (4598)
CMV promoter
p2-3'IL4/EGFP-F
4976 bp
EGFP-F
Eco RI (1428)
Sca I (1447)
IL4 3'UTR
KAN/NEO
Eco RI (1598)
Xba I (1641)
Bsm I (1745)
SV40 polyA signal
SV40 polyA signal
Bsm I (1838)

Fig. 12

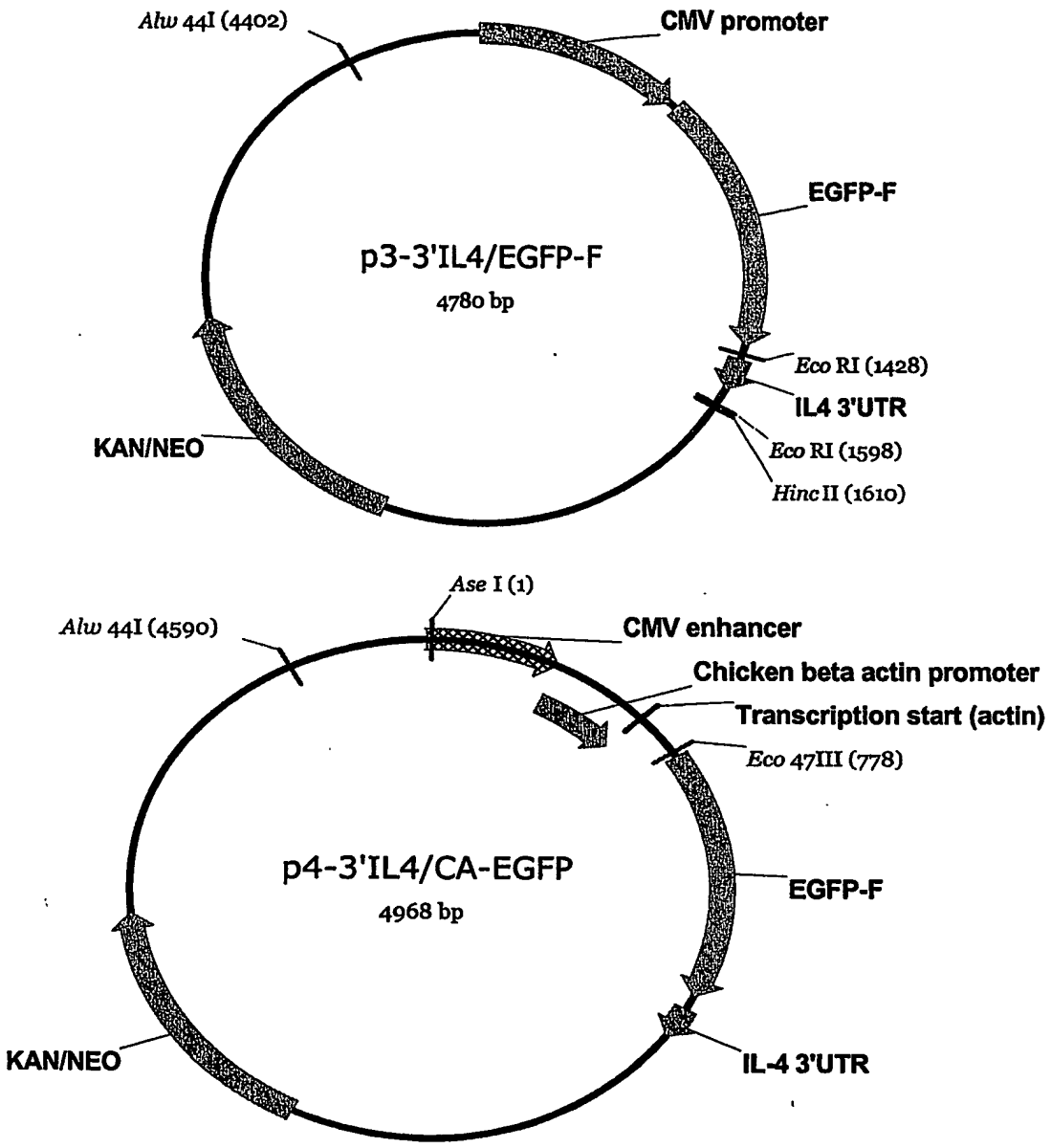

Alw 44I (4402)
CMV promoter
EGFP-F
p3-3'IL4/EGFP-F
4780 bp
Eco RI (1428)
IL4 3'UTR
Eco RI (1598)
Hinc II (1610)
KAN/NEO
Ase I (1)
Alw 44I (4590)
CMV enhancer
Chicken beta actin promoter
Transcription start (actin)
Eco 47III (778)
p4-3'IL4/CA-EGFP
4968 bp
EGFP-F
KAN/NEO
IL-4 3'UTR

Fig. 13

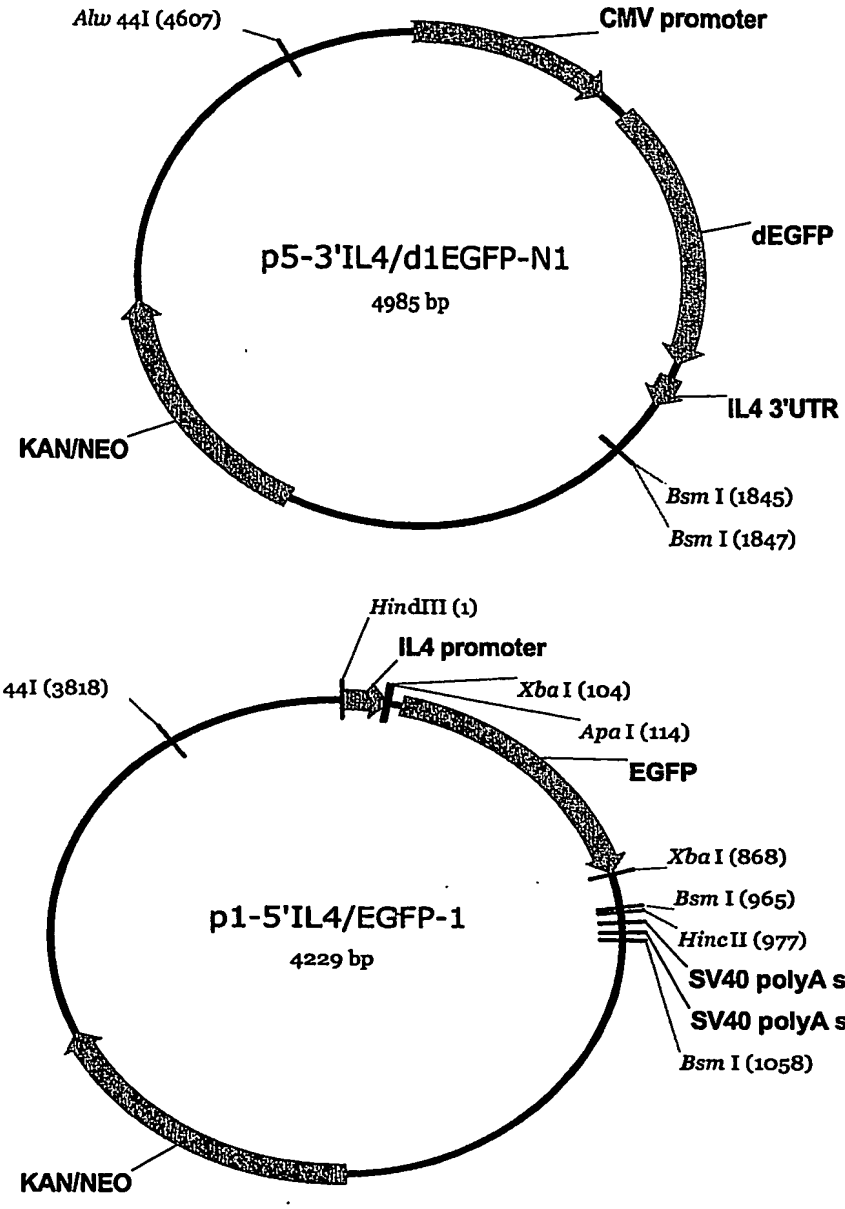

Alw 44I (4607)
CMV promoter
dEGFP
p5-3'IL4/d1EGFP-N1
4985 bp
IL4 3'UTR
KAN/NEO
Bsm I (1845)
Bsm I (1847)
HindIII (1)
IL4 promoter
Alw 44I (3818)
Xba I (104)
Apa I (114)
EGFP
Xba I (868)
Bsm I (965)
p1-5'IL4/EGFP-1
4229 bp
HincII (977)
SV40 polyA signal
SV40 polyA signal
Bsm I (1058)
KAN/NEO

Fig. 14

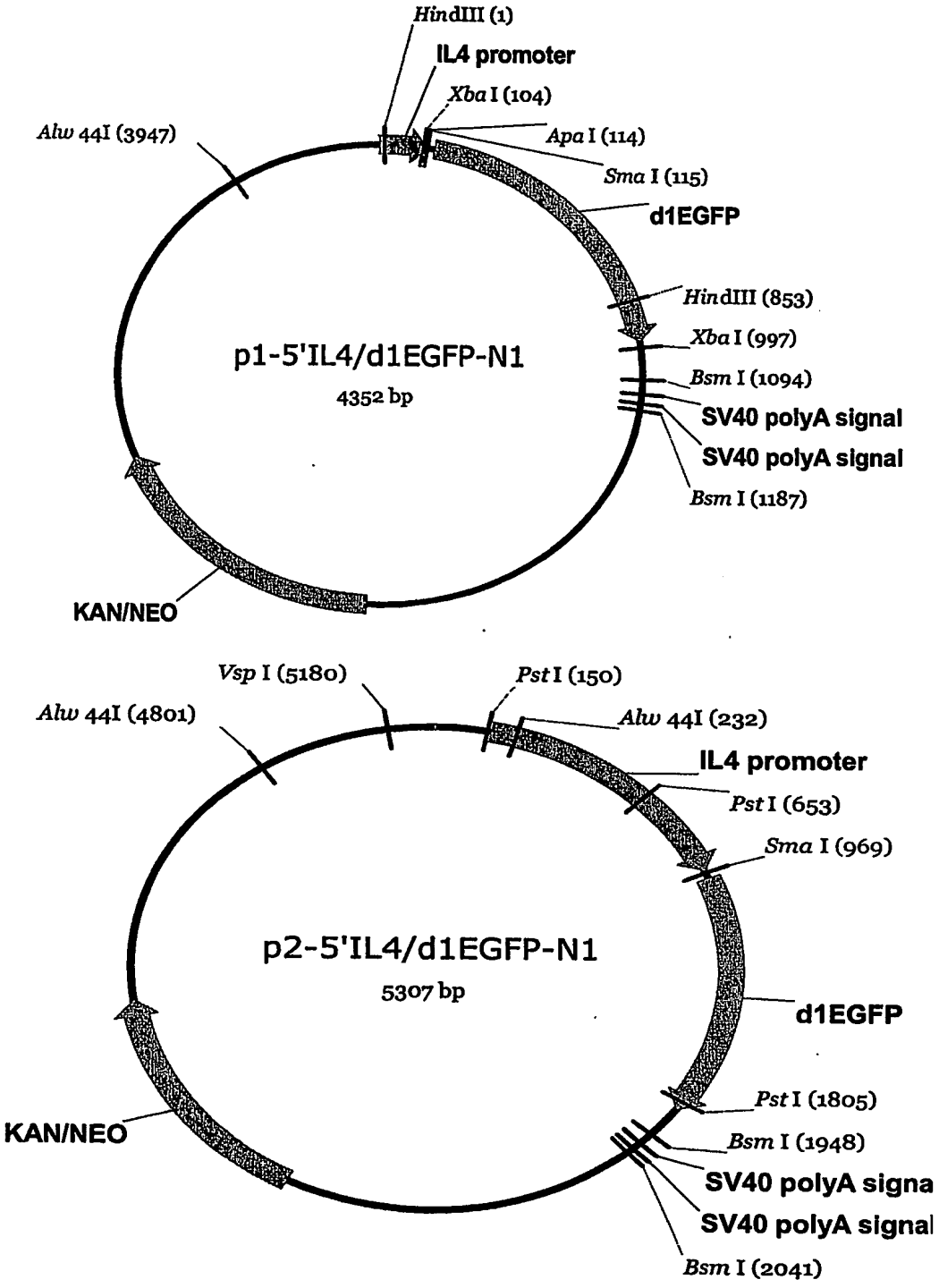

HindIII (1)
IL4 promoter
Xba I (104)
Apa I (114)
Sma I (115)
d1EGFP
HindIII (853)
Xba I (997)
Bsm I (1094)
SV40 polyA signal
SV40 polyA signal
Bsm I (1187)
Alw 44I (3947)
p1-5'IL4/d1EGFP-N1
4352 bp
KAN/NEO
Vsp I (5180)
Pst I (150)
Alw 44I (232)
IL4 promoter
Pst I (653)
Sma I (969)
d1EGFP
Pst I (1805)
Bsm I (1948)
SV40 polyA signa
SV40 polyA signal
Bsm I (2041)
Alw 44I (4801)
p2-5'IL4/d1EGFP-N1
5307 bp
KAN/NEO

Fig. 15

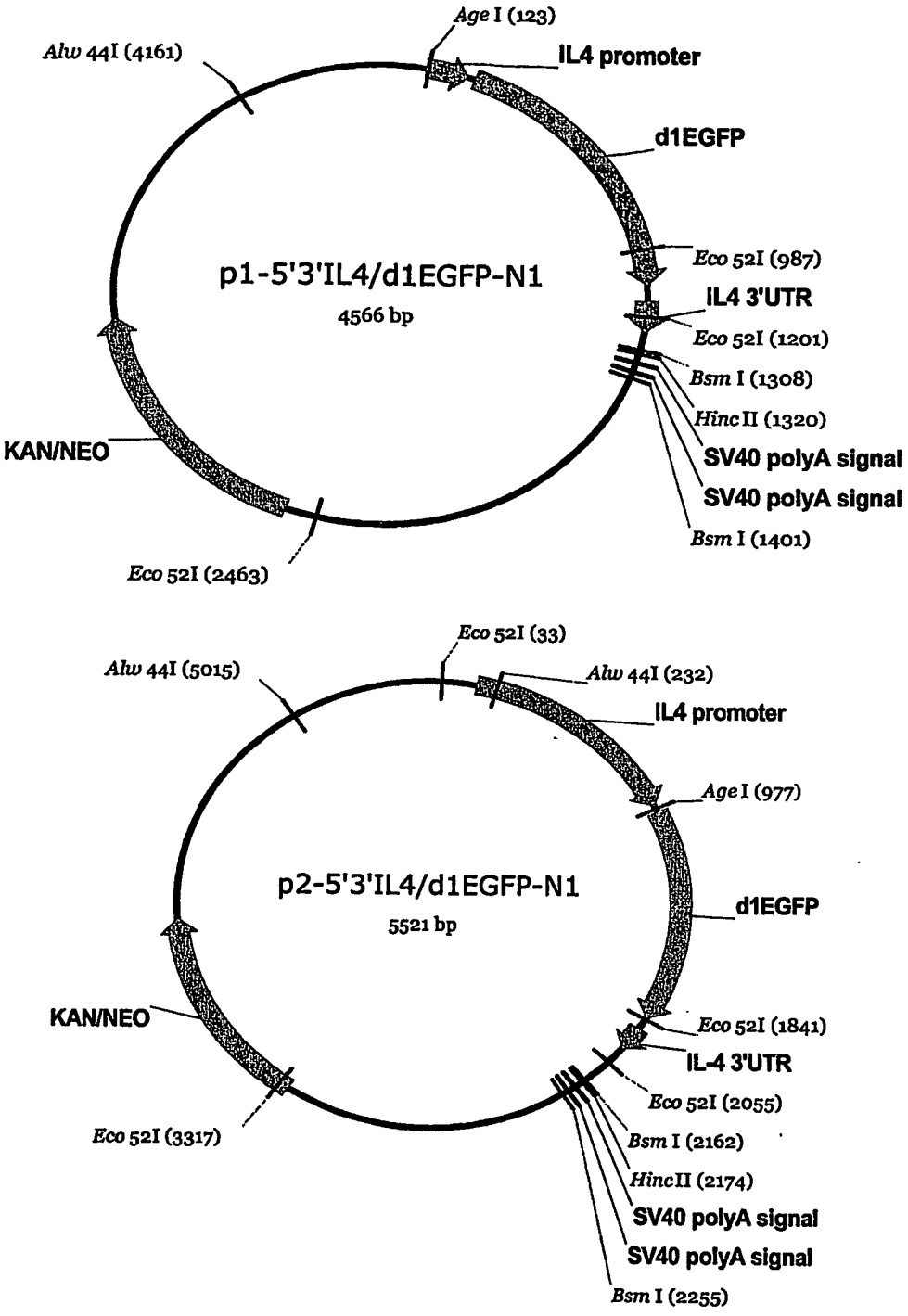

Age I (123)
IL4 promoter
d1EGFP
Eco 52I (987)
IL4 3'UTR
Eco 52I (1201)
Bsm I (1308)
HincII (1320)
SV40 polyA signal
SV40 polyA signal
Bsm I (1401)
Alw 44I (4161)
p1-5'3'IL4/d1EGFP-N1
4566 bp
KAN/NEO
Eco 52I (2463)
Eco 52I (33)
Alw 44I (232)
IL4 promoter
Age I (977)
d1EGFP
Eco 52I (1841)
IL-4 3'UTR
Eco 52I (2055)
Bsm I (2162)
HincII (2174)
SV40 polyA signal
SV40 polyA signal
Bsm I (2255)
Alw 44I (5015)
p2-5'3'IL4/d1EGFP-N1
5521 bp
KAN/NEO
Eco 52I (3317)

Fig. 16

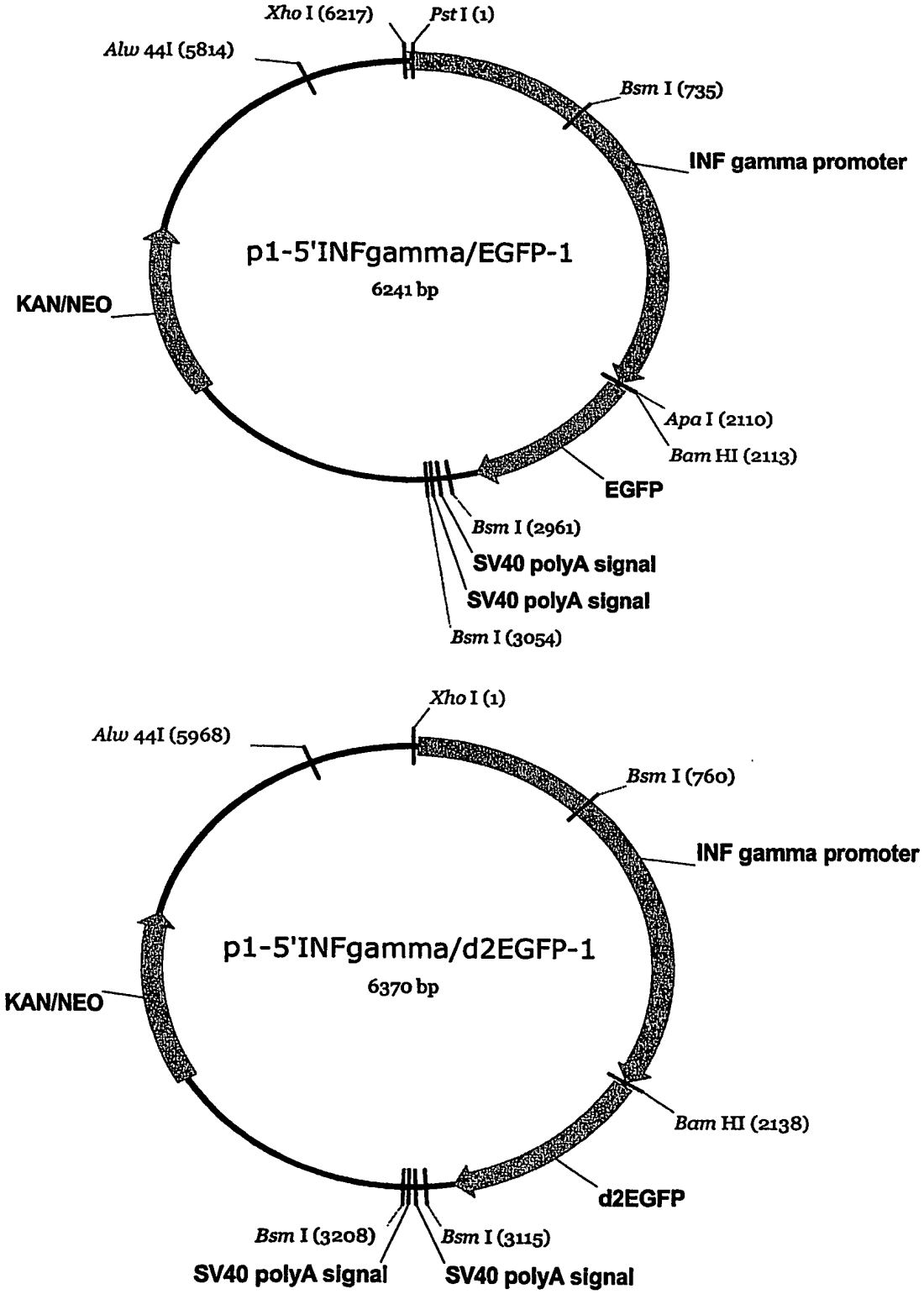

Xho I (6217)
Pst I (1)
Alw 44I (5814)
Bsm I (735)
INF gamma promoter
p1-5'INFgamma/EGFP-1
6241 bp
KAN/NEO
Apa I (2110)
Bam HI (2113)
EGFP
Bsm I (2961)
SV40 polyA signal
SV40 polyA signal
Bsm I (3054)
Xho I (1)
Alw 44I (5968)
Bsm I (760)
INF gamma promoter
p1-5'INFgamma/d2EGFP-1
6370 bp
KAN/NEO
Bam HI (2138)
d2EGFP
Bsm I (3208)
Bsm I (3115)
SV40 polyA signal
SV40 polyA signal

Fig. 17

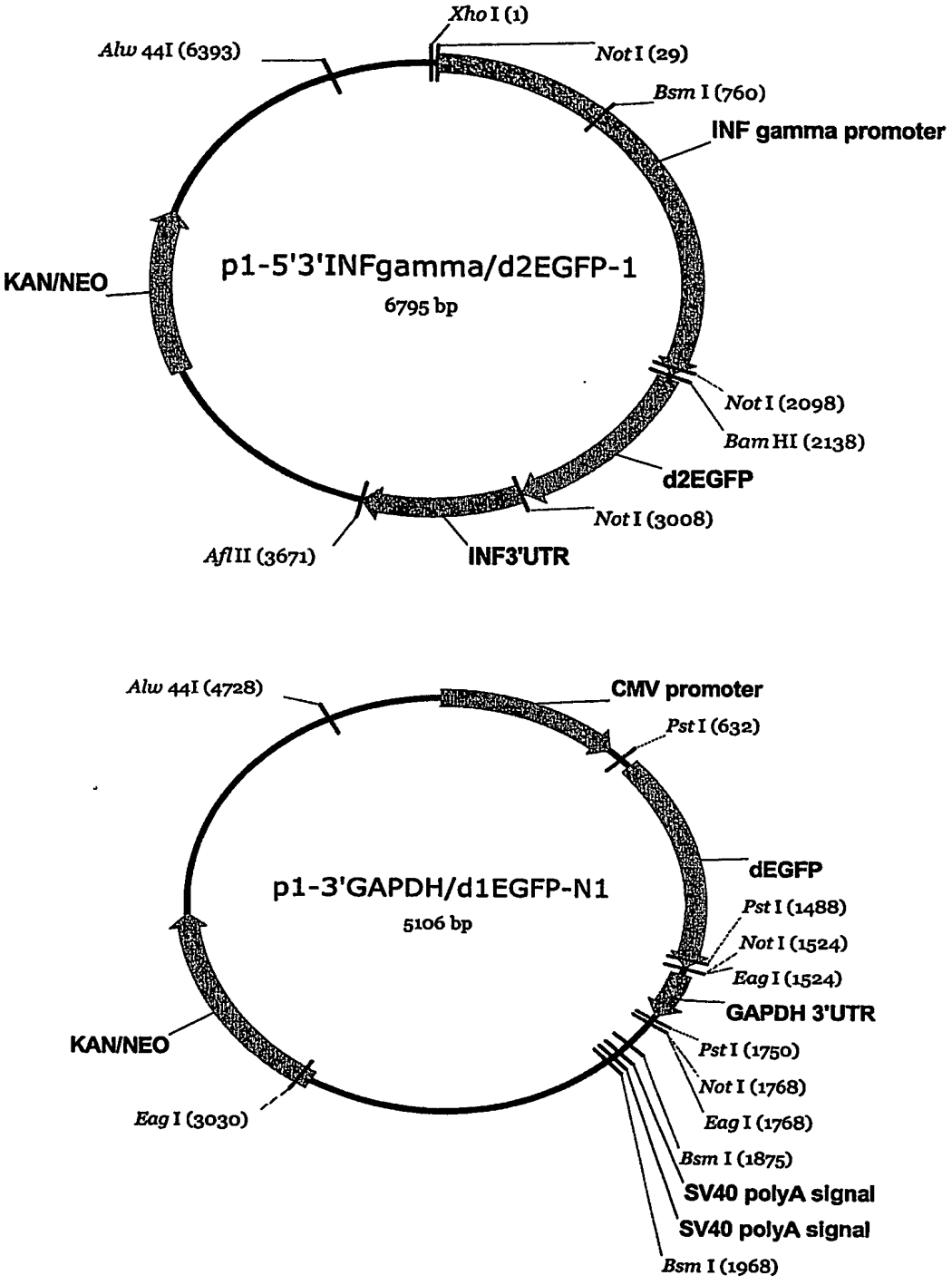

Xho I (1)
Not I (29)
Bsm I (760)
INF gamma promoter
Alw 44I (6393)
p1-5'3'INFgamma/d2EGFP-1
6795 bp
KAN/NEO
Not I (2098)
Bam HI (2138)
d2EGFP
Not I (3008)
Afl II (3671)
INF3'UTR
Alw 44I (4728)
CMV promoter
Pst I (632)
dEGFP
p1-3'GAPDH/d1EGFP-N1
5106 bp
Pst I (1488)
Not I (1524)
Eag I (1524)
GAPDH 3'UTR
KAN/NEO
Pst I (1750)
Not I (1768)
Eag I (1768)
Eag I (3030)
Bsm I (1875)
SV40 polyA signal
SV40 polyA signal
Bsm I (1968)

Fig. 18

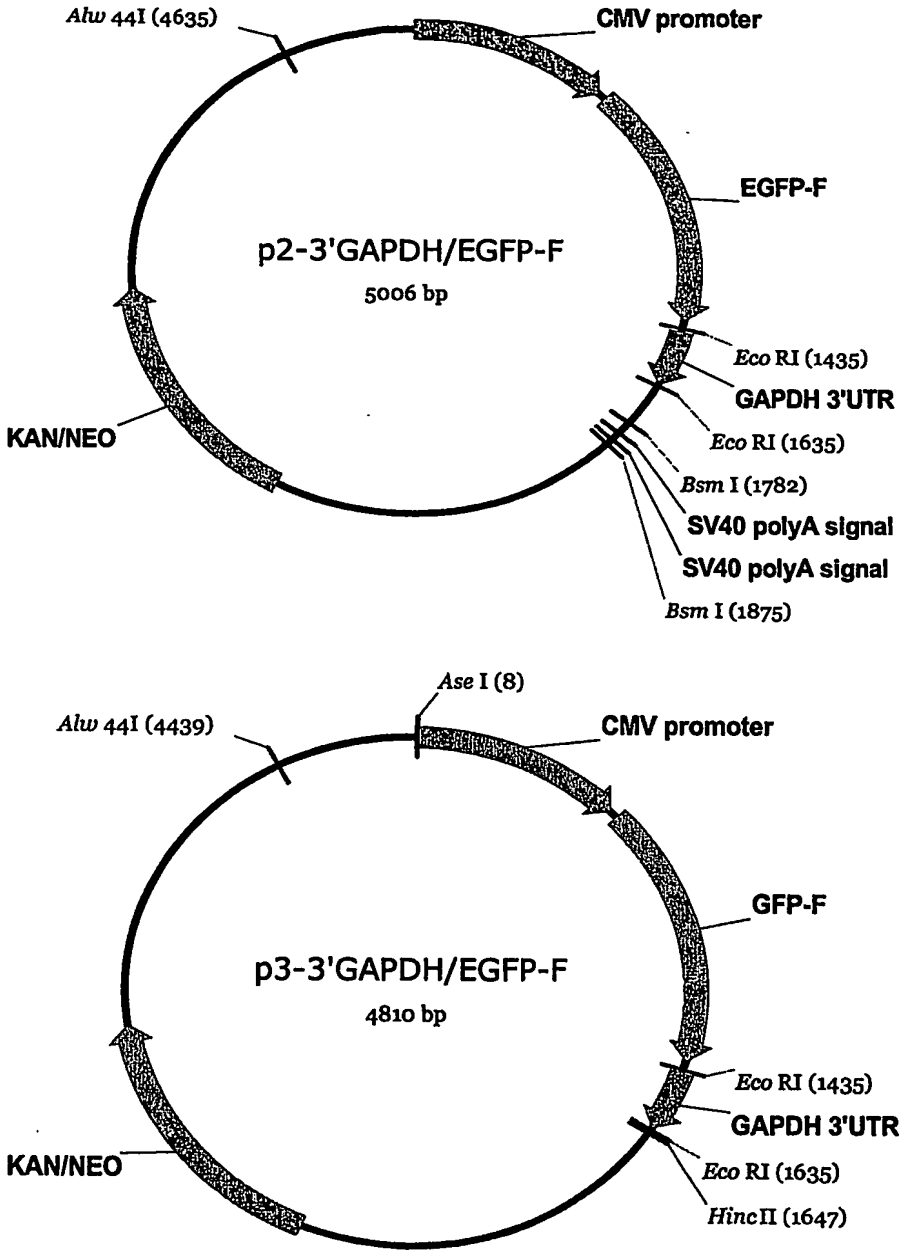

Alw 44I (4635)
CMV promoter
EGFP-F
p2-3'GAPDH/EGFP-F
5006 bp
Eco RI (1435)
GAPDH 3'UTR
Eco RI (1635)
Bsm I (1782)
SV40 polyA signal
SV40 polyA signal
Bsm I (1875)
KAN/NEO
Ase I (8)
Alw 44I (4439)
CMV promoter
GFP-F
p3-3'GAPDH/EGFP-F
4810 bp
Eco RI (1435)
GAPDH 3'UTR
Eco RI (1635)
HincII (1647)
KAN/NEO

Fig. 19

Fig. 20

Cell viability testing.
Cell lines employed in the project were exposed to incresing concentrations of chemicals listed in Table II. LDH relase was determined using test (Roche) according to the manufacturer protocol. All concentrations are expresed in [µM].

		Hel 30	3T3-L1+pEGFFP-F	EL-4	J774A1
Chemical	**solvent**	**Karcinocyte**	**Fibroblast**	**T-cell leukemia**	**Macrophage-monocyte**
Benzocaine CAS Nr: 94-09-7	Ethanol	A: - B: 3000, 1000, 100 C: 10 D: -	A: - B: 3000, 1000 C: 100, 10 D: -	A:- B:- C: 1500 D: 1000, 100	A: 3000 B: 1000 C:- D:100, 10
Cyclosporin	Ethanol	A: - B: 10 C: 1, 0.1, 0.01 D: -	A: - B: 30, 10 C: 1, 0.1 D: -	A: 15, 10 B: 1 C:- D:-	A: 30, 10 B: - C: - D: 1, 0.1
DNCB, dinitrochlorobenzene CAS Nr: 9700-7	Ethanol	A: 100, 33, 10 B: - C: 3,3 D: -	A: 100, 10, 3.3 B: - C: 1 D: -	A: 10 B: - C: - D: 1, 0.33, 0.033	A: 10, 5 B: - C: - D: 0.5, 0.05
MDI,diphenylmethane-4,4-diisocyanate CAS Nr: 101-68-8	DMSO	A: - B: - C: 1000, 100, 10, 1 D: -		A: 1500 B: - C: - D: 150, 15, 1.5	
HgCl2, mercuric chloride Cas nr: 7487-94-7	ethanol	A: - B: - C: 1, 0.1, 0.01, 0.001 D: -	X: 10 A: - B: - C: 1, 0.1, 0.01 D: -	X: 10 A: 30 B: - C: - D: 1, 0.01	

Fig. 20 (continuation)

Chemical	solvent	Hel 30 Karcinocyte	3T3-L1+pEGFFP-F Fibroblast	EL-4 T-cell leukemia	J774A1 Macrophage-monocyte
Penicillin G Cas nr: 140-64-7	medium	A: - B: - C: 1000, 100, 10, 1 D: -	A: - B: - C: 1000, 100, 10, 1 D: -	A: - B: - C: - D: 1000, 100, 10, 1	
SDS, sodium dodecyl sulphate	DMSO	A: 500 B: - C: 50, 5, 0.5 D: -	A: 500 B: - C: 50, 5, 0.5 D: -	A: 250 B: - C: - D: 50, 5, 0.5	
TBTO, bis-tributyltin oxide Cas nr: 584-3-9	ethanol	A: 100, 10, 1 B: 0.1 C: - D: -	A: 100, 10, 1 B: - C: 0.1 D: -	A: 10, 1, 0.5, 0.1, 0.05 B: 0.1 C: - D: -	
TDI, toluene-2,4-diisocyanate Cas nr: 584-84-9	ethanol	A: 1500 B: 150 C: 15, 1.5 D: -		A: B: 1000 C: D: 100, 10, 1	
K2PtCl4, tetrachloroplatinate (platinum salt) Cas nr: 10025-99-7	medium	A: - B: - C: 8.6, 0.86, 0.086, 0.0086 D: -	A: 300, 100 B: - C: 10, 1 D: -	A: B: 150, 50 C: D: 5, 0.5	
Thalidomide, cas nr 50-35-1	DMSO	C: 1000, 100, 10, 1	C: 1000, 100, 10, 1	D: 1000, 100, 10, 1	

A: toxic concentration, B: concentration inhibits cell growth, no toxicity, C: no toxic effect or growth inhibition, X: lower amount of LDH, but no toxic effect or cell growth inhibition

Fig. 21

Comparision of data obtained in cytotoxicity tests in two participating laboratories
Direct cytotoxicity assciated with chemicals listed in Table II was determined for EL-4 cell line using LDH relase assay in two laboratories according to the same experimental protocol. All concentrations are expresed in [μM].

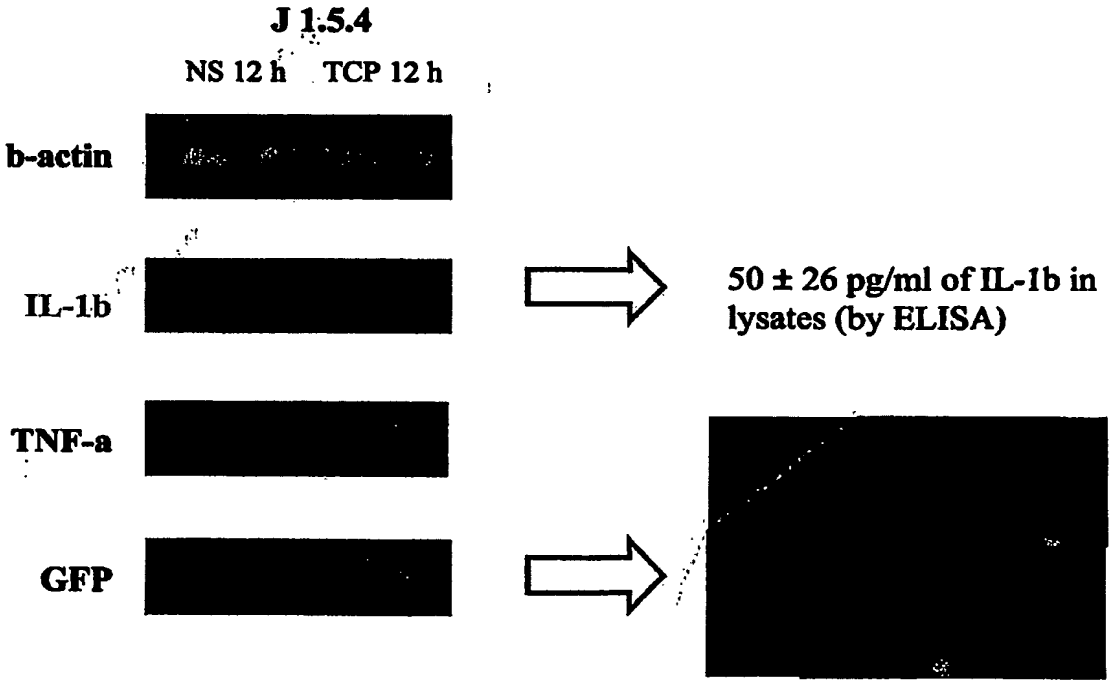
			EL-4	
Chemical	**solvent**	**NIPH solv.**	**T-cell leukemia**	**in NIPH**
Cyclosporin	Ethanol	Ethanol	A: 15, 10 B: 1 C:- D:-	20ug/mL and above is Toxic below 10ug/mL OK
Penicillin G Cas nr: 140-64-7	medium		A: - B: - C: - D: 1000, 100, 10	2500 U = 15 mg/mL and below not toxic
Pentamidine cas nr: 140-64-7	medium	DMSO		30ug/mL and below=OK, 60ug/mL usure, 80ug/mL and above toxic
Rapamycin cas nr 53123-88-9		DMSO		Think 25000ng/mL toxic, below 1000 ng/mL OK
SDS, sodium dodecyl sulphate	DMSO	water	A: 1500 B: - C: - D: 150, 15, 1.5	50ug/mL and below not toxic, 2,5 mg/mL toxic
HgCl2, mercuric chloride Cas nr: 7487-94-7	ethanol		X: 10 A: 30 B: - C: - D: 1, 0.01	
TBTO, bis-tributyltin oxide Cas nr: 584-3-9	ethanol			
TDI, toluene-2,4-diisocyanate Cas nr: 584-84-9	ethanol			
K2PtCl4, tetrachloroplatinate (platinum salt) Cas nr: 10025-99-7	medium	water		150uM = - 20 % toxicity
Thalidomide, cas nr 50-35-1	DMSO			

Fig. 22

Expression of IL-1β and GFP in J.1.5.4 stimulated with tetrachloroplatinate TCP

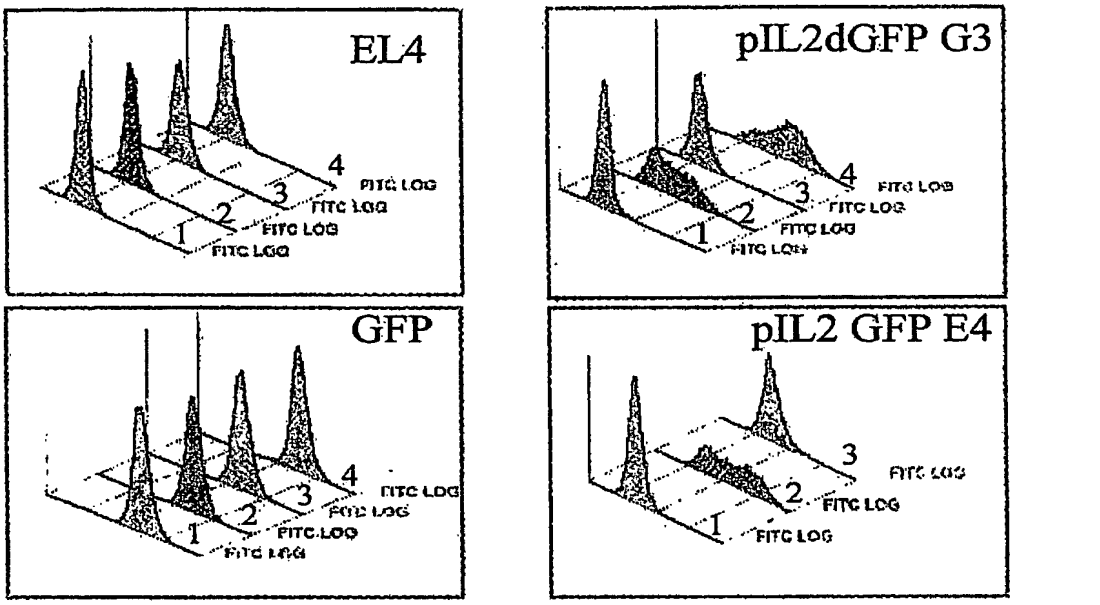
A. The EC_{50} values for selected chemicals from the list of model immunotoxicants (concentration causing death of 50% of cells in the population) obtained with MTT assay with macrophages J774A.1 and clone J 1.5.4.

B. J 1.5.4. reporter cells were incubated with these chamicals and observed under fluorescence microscope. In the case of tetrachloroplatinate upregulation of green fluorescence was observed. The expression of GFP and endogenous IL-1β was confirmed with RT-PCR and with RT-PCR and ELISA, respectively.



Response of reporter cell lines to model xenobiotics (I)

Two EL-4 derived IL-2 expresion reporter cell lines were activated with TPA/ionomycine for 16 hr in the presence or absence of cyclosporin A or Rapamycin. The level of EGFP mediated fluorescence was determined by FACS



1 - control (only medium) 3 - ionomycin/PMA + CsA (2 ug/mL)

2 - ionomycin (1uM) + PMA (10 ng/mL) 4 - ionomycin/ PMA + Rapamycin (20 ng/mL)

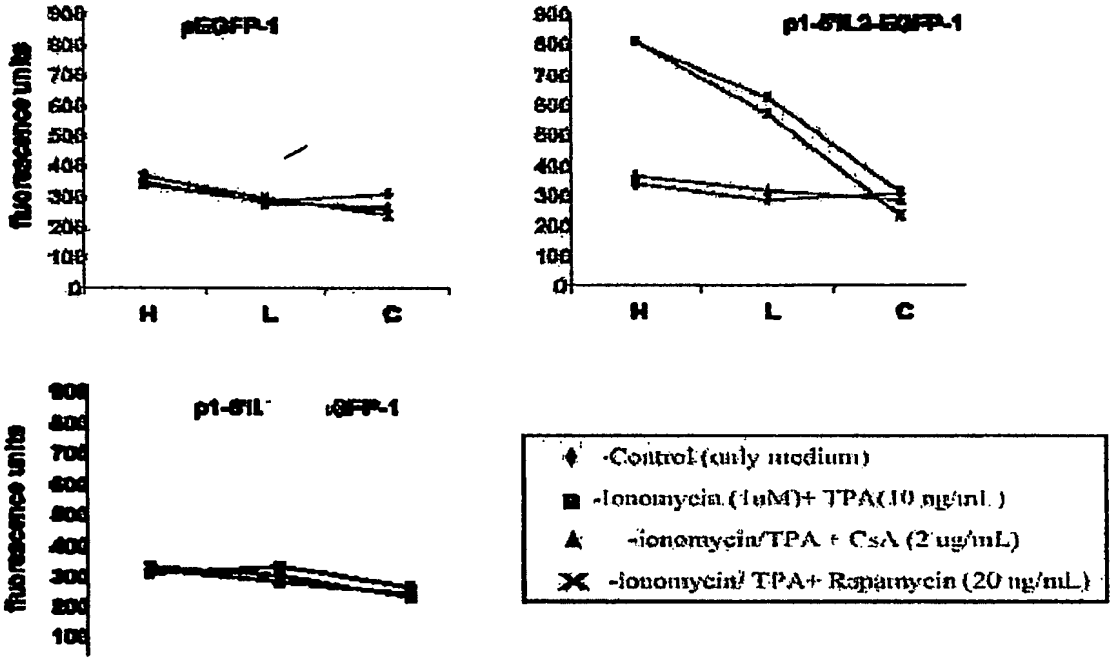
Fig. 23

Fig. 24

Response of reporter cell lines to model xenobiotics (II)

Two EL-4 derived IL-2 expression reporter cell lines were activated with TPA/ionomycine for 16 hr in the presence or absence of Cyclosporin A or Rapamycin.. The level of ECFP mediated fluorescence was determined with Fluorostar plate reader (A) and under fluorescence microscope (B)

A



B

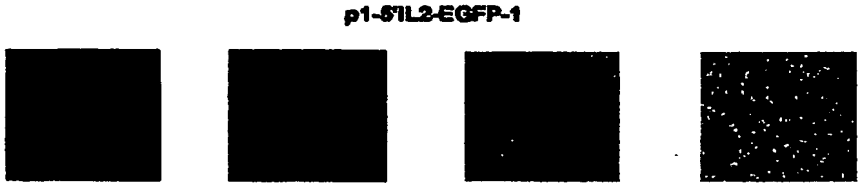


Fig. 25

Response of reporter cell lines to model xenobiotics (III)

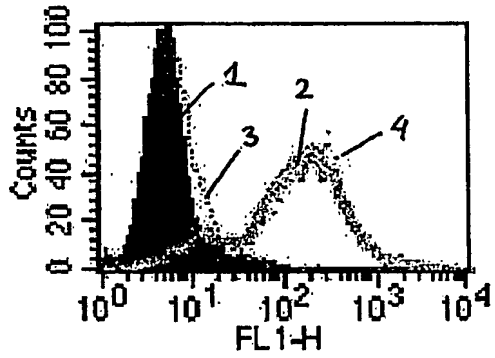
A. EL-4 cells were incubated were activated with TPA/ionomycine for 16 hr in the presence or absence of Cyclosporin A or Rapamycin or TCDD. RNA was isolated using Tri reagent and RTPCR using primers specific for IL-2and GAPDH (control) were performed. PCR products were analyzed on agarose gel

B EL-4 derived reporter cells were incubated with media alone or activated with TPA/ionomycine for 16 hr in the presence or absence of Cyclosporin A or Rapamycin for 16 hr and the level of EGFP mediated fluorescence was determined by FACS.

A

M: size marker
1: control
2: TPA/ionomycin
3: TPA/ionomycin + CsA
4: TPA/ionomycin + Rap.
5: TPA/ionomycin + TCDD

B



1 Control
2 TPA/ionomycin
3 TPA/ionomycin + CsA
4 TPA/ionomycin + Rap.

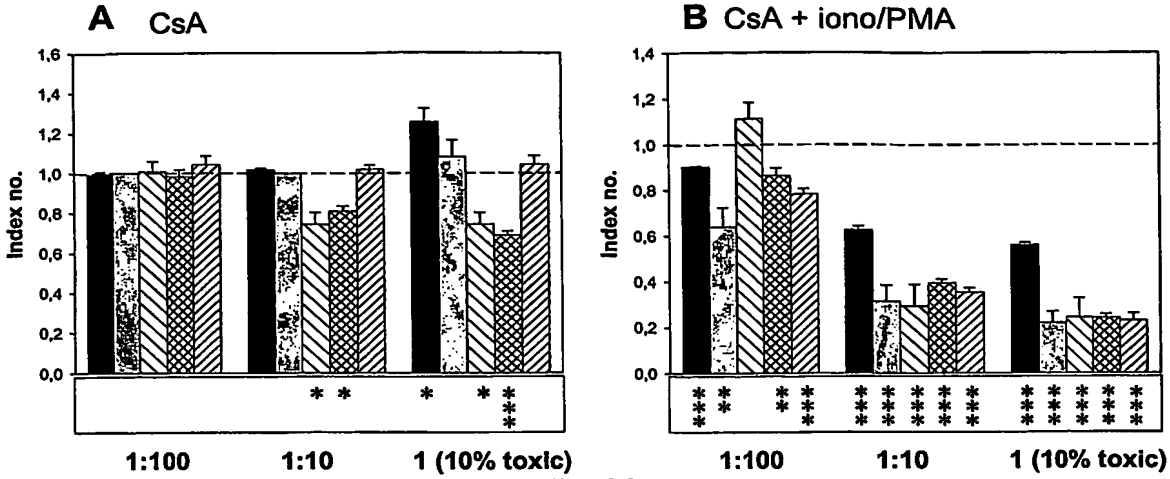


Fig. 26

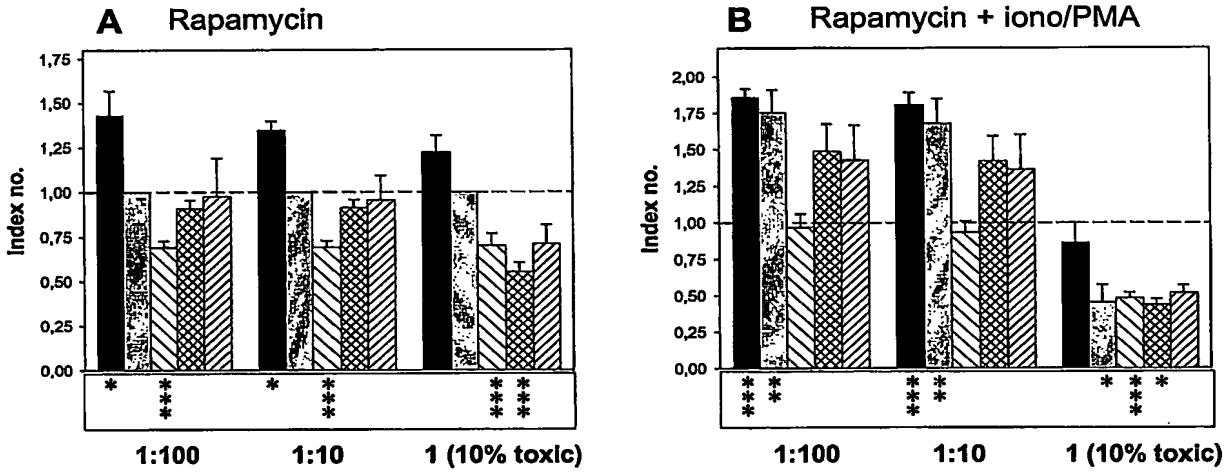


Fig. 27

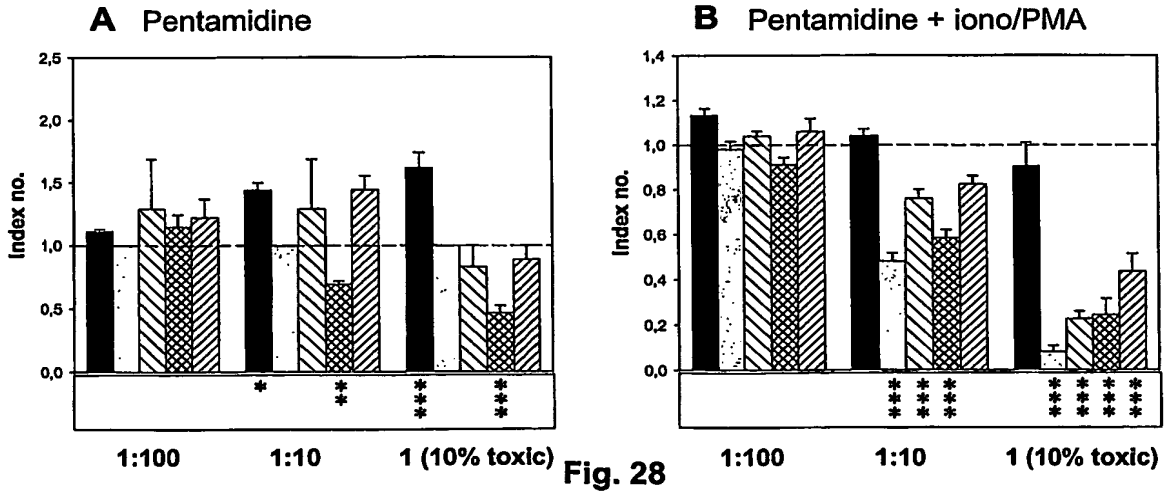


Fig. 28

Fig. 29

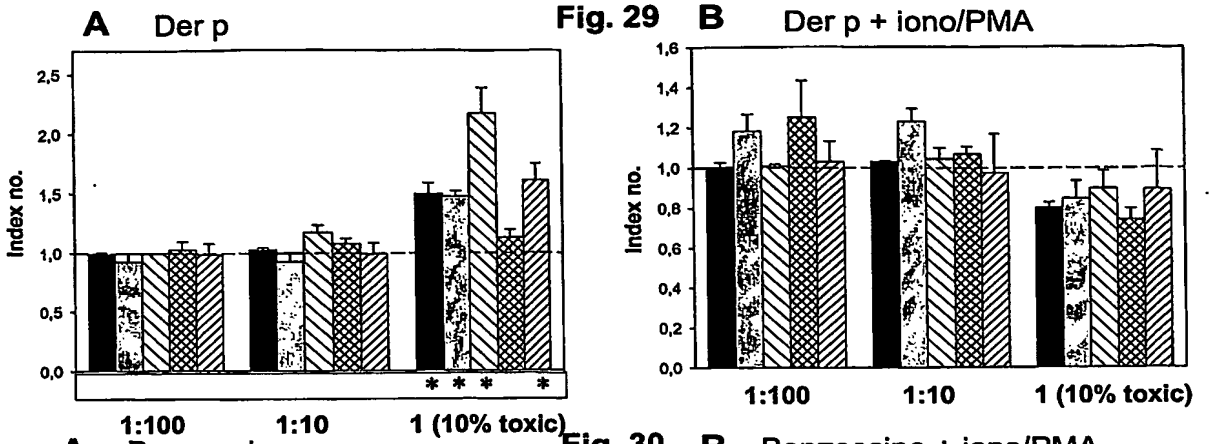


Fig. 30

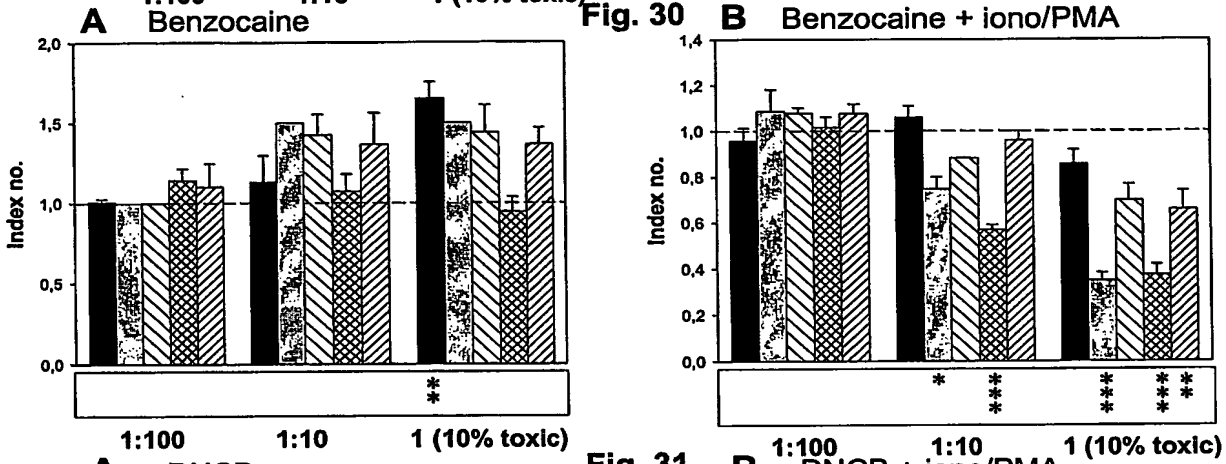


Fig. 31

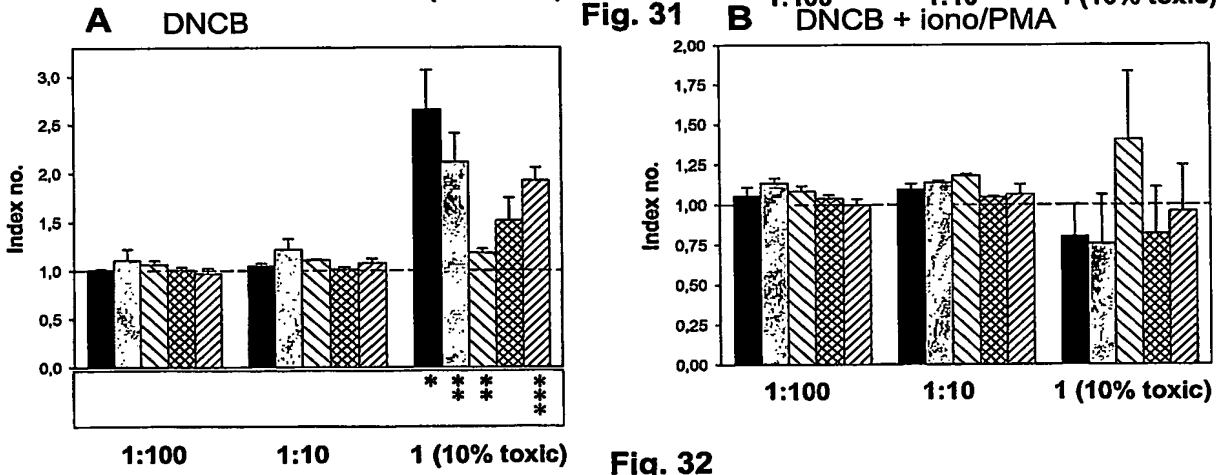


Fig. 32